Comparative Analysis of Cobra Venom and LPS-induced Inflammatory Responses in Mammalian Cell Models

Regina Veloz[1,2], Martha Barrientos[1,3], Elda E. Sánchez[1,3], Emelyn Salazar[1,2]

[1]National Natural Research Center (NNTRC), Texas A&M University Kingsville, Kingsville, TX, USA
[2]Department of Biological and Health Sciences, Texas A&M University-Kingsville, Kingsville, TX, USA.
[3]Department of Chemistry , Texas A&M University Kingsville, Kingsville, TX, USA

TEXAS A&M UNIVERSITY KINGSVILLE
McNAIR SCHOLARS PROGRAM

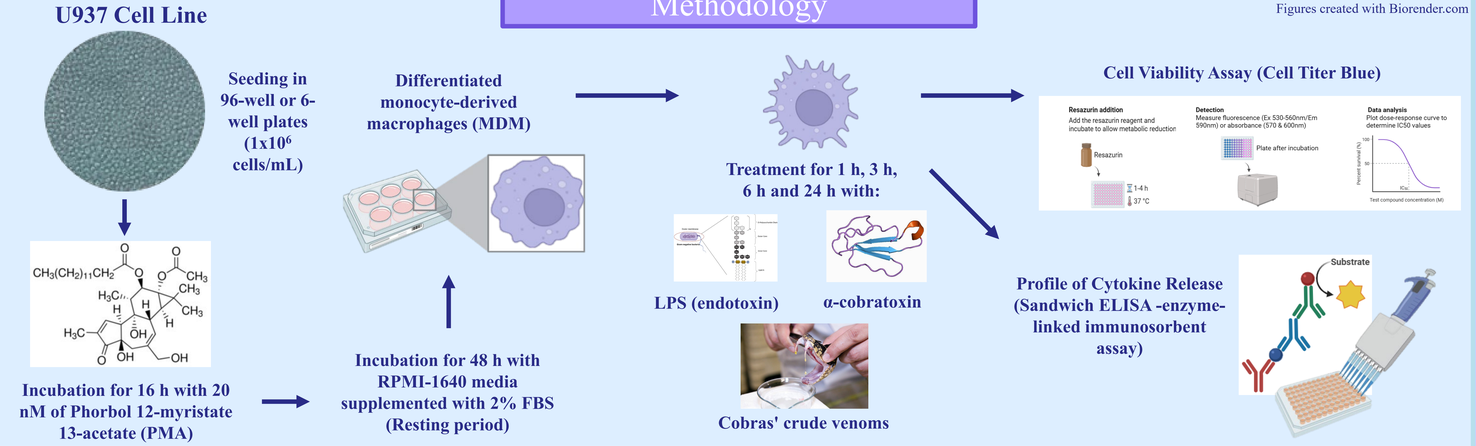
NATIONAL NATURAL TOXINS RESEARCH CENTER (NNTRC)

Abstract

Snake envenomation poses a significant public health concern, yet the mechanisms of venom-induced morbidity remain poorly studied. While current antivenom therapies neutralize lethal effects, they often fail to address local tissue damage and persistent inflammation. This study investigates the innate immune response to cobra venom by comparing its inflammatory effects to those of lipopolysaccharide (LPS), a well-characterized bacterial endotoxin. We analyzed how crude venoms from *Naja melanoleuca* and *Naja kaouthia*, and purified α-cobratoxin from *N. kaouthia* modulate the immune response in human monocyte-derived macrophages (MDMs), obtained after differentiation of the U937 cell line. Cell viability was first assessed after 24 h of treatment using the Cell-Titer Blue assay. After determining the IC_{50}, non-cytotoxic concentrations were used to evaluate cell activation. At 1 h, 3 h, 6 h and 24 h post-treatment, cell supernatants were collected and the main cytokines (tumor necrosis factor-alpha, TNF-α,interleukin-6, IL-6, interleukin-8, IL-8, and interleukin-10, IL-10) were measured using enzyme-linked immunosorbent assay (ELISA). Our results showed that *N. melanoleuca* venom caused the highest cytotoxicity and elicited a strong pro-inflammatory response, with cytokine release levels comparable to LPS-treated cells. In contrast, *N. kaouthia* venom and α-cobratoxin triggered only mild to low inflammatory responses. These findings reveal both shared and toxin-specific immune signatures, advancing our understanding of the immunomodulatory potential of cobra venoms. Uncovering these mechanisms may contribute to the development of improved treatments for venom-induced inflammation.

Methodology

Figures created with Biorender.com



Introduction

Snakebite envenomation is a major yet under-researched public health issue, especially in tropical areas where access to antivenom care is limited[1] (Fig.1). While antivenom neutralizes circulating toxins, it often fails to resolve local tissue damage and long-term inflammation[2]. This project explores early immune responses triggered by cobra venom in comparison to lipopolysaccharide (LPS), a well-studied bacterial endotoxin commonly used as a pro-inflammatory control[3]. By comparing how crude venom from *Naja melanoleuca* and *Naja kaouthia* (Fig. 2), as well as purified α-cobratoxin—the main neurotoxin found in *N. kaouthia* venom—affect cytokine production in human-derived macrophages, we aim to better understand the inflammatory mechanisms behind venom-induced immune activation. Understanding these processes may help identify why snakebites cause prolonged damage even after antivenom is administered. This research is critical because studying the effects of cobra venoms provides insight into the immunopathology of envenomation and can guide future therapeutic strategies[4,5].

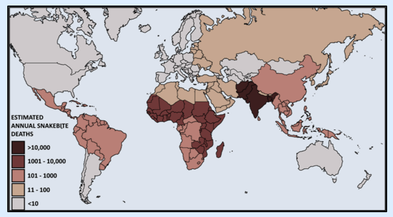


Figure 1. Map of estimated annual snakebite deaths with the highest rates occurring in sub-Saharan Africa and South Asia.

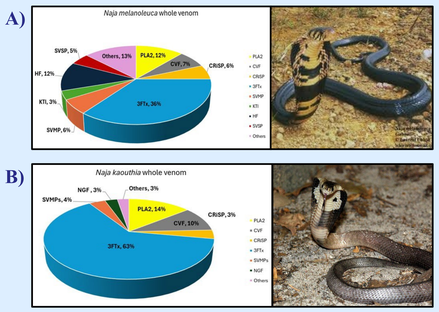
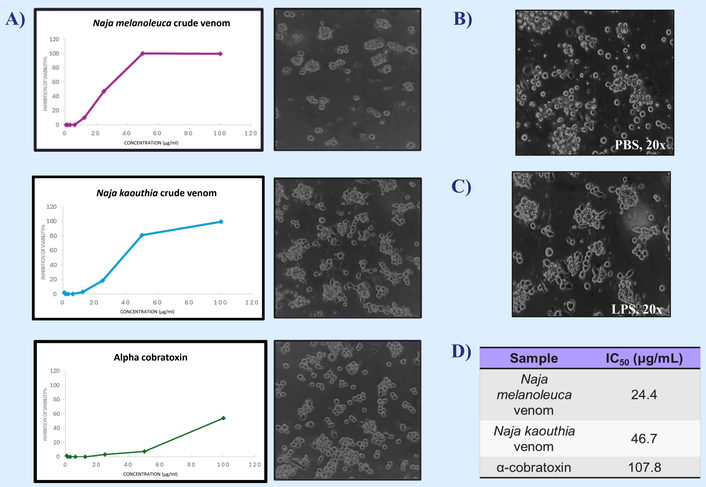


Figure 2. A) Venom composition and image of *Naja melanoleuca* (Forest cobra). **B)** Venom composition and image of *Naja kaouthia* (Monocled cobra).

Results



Sample	IC_{50} (µg/mL)
Naja melanoleuca venom	24.4
Naja kaouthia venom	46.7
α-cobratoxin	107.8

Figure 3. Effect of cobra venoms and toxins on cell viability. A) Inhibition of cell viability on U937 MDM cells treated for 24 h with various concentrations of *N. melanoleuca* whole venom, *N. kaouthia* whole venom and α-cobratoxin were tested using Cell-titer blue assay. Results were expressed as the percentage of inhibition of cell viability relative to the negative control of cells treated with PBS. **B)** PBS: Negative control, cells treated with vehicle only. **C)** LPS: Cells treated with 0.25 µg/mL lipopolysaccharide. **D**) Inhibitory concentration 50 (IC_{50}) of the three treatments for U937-MDM cells.

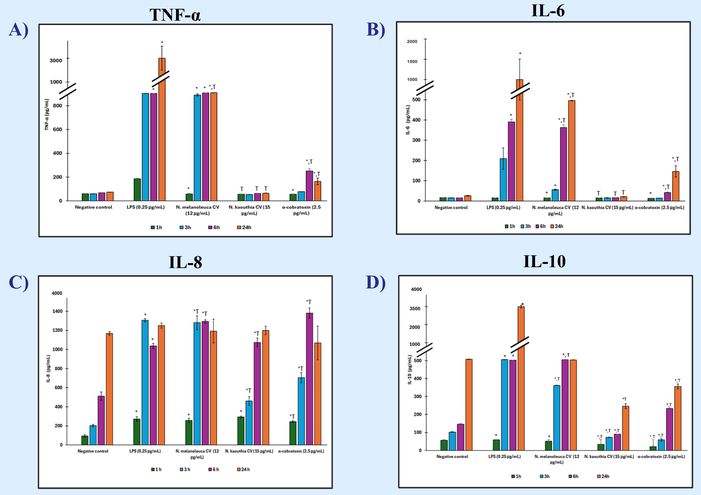


Figure 4. Release of cytokines on U937- MDM. Cells were treated for 1 h, 3 h, 6 h, and 24 h with non-cytotoxic concentrations of *N. melanoleuca* whole venom, *N. kaouthia* whole venom, and α-cobratoxin. Supernatants were collected and the main pro-inflammatory cytokines TNF-α (A), IL-6 (B) and IL-8 (C) , and the anti-inflammatory cytokine IL-10 (D) were determined using ELISA commercial kits. Cytokine release was expressed as pg/mL. Positive control: LPS (lipopolysaccharide). Negative control: untreated cells. *$p < 0.05$ vs. negative control of cells treated with the vehicle only; $^{T}p< 0.05$ vs. positive control of cells treated with LPS.

Conclusion

- *Naja melanoleuca* venom induced the most significant cytotoxicity among all treatments in human macrophages, with and IC_{50} of 24.4 µg/mL.
- *N. melanoleuca* venom induced a strong pro-inflammatory immune response, promoting the release of TNF-α, IL-6, and IL-8, suggesting it shares similar immune activation patterns with LPS.
- In contrast, *Naja kaouthia* venom and α-cobratoxin triggered only moderate to minimal immune responses, indicating potential differences in venom-related immune-modulating mechanisms.
- Our results highlight that venomous snakes belonging to the same genus can produce distinct inflammatory signatures, emphasizing the need for detailed venom characterization.
- Understanding these unique immune profiles not only provides insights into the pathophysiology of snake envenomation but may also contribute to the development of targeted therapies for venom-induced inflammation.

References

1. Gutiérrez, J. M., Escalante, T., Rucavado, A., & Herrera, C. (2017). Hemorrhage caused by snake venom metalloproteinases: A journey of discovery and understanding. Toxins, 9(10), 307. https://doi.org/10.3390/toxins9100307
2. Hsu, C. H., Chiu, W. T., & Chang, Y. C. (2016). Human monocyte differentiation and cytokine response to lipopolysaccharide. Journal of Immunology Research, 2016, 8261645. https://doi.org/10.1155/2016/8261645
3. Rhee, S. G. (2014). Cell signaling: H_2O_2, a necessary evil for cell signaling. Science, 312(5782), 1882–1883. https://doi.org/10.1126/science.1126783
4. Teixeira, C. F. P., & Zamuner, S. R. (2019). Inflammatory effects of snake venom metalloproteinases. Memórias do Instituto Oswaldo Cruz, 114, e190100. https://doi.org/10.1590/0074-02760190100
5. Zuliani, J. P., Soares, A. M., & Gutiérrez, J. M. (2023). Role of cytokines and inflammatory mediators in snakebite envenoming. Toxicon, 224, 107035. https://doi.org/10.1016/j.toxicon.2023.107035

Acknowledgments

- McNair Scholars Program
- Funding for the project was provided by: NIH/ORIP, Viper Resource Grant #P40OD01960-21 (Dr. E.E. Sánchez).
- We want to thank Juan Salinas, and the rest of the NNTRC personnel for their assistance.

TRIO RONALD E. MCNAIR POST-BACCALAUREATE ACHIEVEMENT PROGRAM

NIH National Institutes of Health